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14. ABSTRACT:
AIB1 (Amplified In Breast Cancer 1) is a nuclear receptor coactivator whose gene is amplified in 5-10% of breast cancers and both the mRNA and protein are overexpressed in ~30% of breast tumors. In vitro studies show that AIB1 plays a significant role in estrogen and IGF-1-induced cell proliferation. Germline knockout of the AIB1 gene leads to reduced somatic growth, abnormal reproductive function and reduced mammary gland development. Knockout of AIB1 expression also abrogates Ras-induced tumorigenesis. Furthermore, patients with tumors expressing high levels of the growth factor HER2/Neu in addition to AIB1 often develop anti-estrogen resistance to tamoxifen therapy. These findings imply that AIB1 plays a fundamental role in the development of hormone-independent breast cancer through growth factor mediated pathways. Nonetheless, the underlying mechanism of AIB1 regulation of growth factor mediated mammary neoplasia is unknown. In this investigation, I will utilize the MMTV-Neu mouse model (develop mammary gland tumors in 7-9 months) to elucidate the specific role of AIB1 in growth factor-induced mammary tumorigenesis.

15. SUBJECT TERMS
None provided.

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INTRODUCTION

The nuclear receptor coactivator AIB1 (**A**mplified **I**n **B**reast **C**ancer **1**), belongs to the p160/SRC steroid receptor coactivator (SRC) family which includes SRC-1[1], TIF-2 (GRIP1) [2], and AIB1 (ACTR/RAC3/TRAM-1/SRC-3) [3]. AIB1 associates with hormone-bound nuclear receptors including ER, PR, GR, RAR and PPAR, and facilitates the transcriptional activation function of the receptors. The AIB1 gene was first implicated in breast cancer when it was shown to be amplified on a region of chromosome 20q, a region often amplified in breast cancer [4]. Furthermore, the AIB1 gene is amplified in 5-10% of breast cancers and both the mRNA and protein are overexpressed in ~30% of breast tumors and breast cancer cell lines [5, 6]. Recent findings have shown that the loss of AIB1 in MMTV/v-Ha-ras mice (inclined to develop mammary cancer) suppresses mammary gland (MG) ductal hyperplasia and tumorigenesis [7] and AIB1 (p/CIP in mice) knockout mice exhibit stunted growth and decreased IGF-1 levels [8]. In addition, AIB1 overexpression correlates with the absence of ER and PR but positively correlates with p53 and HER2/Neu signaling [9], implying that in a subset of breast tumors, AIB1 may be interacting through hormone-independent signaling pathways. Moreover, a high level of AIB1 protein is associated with high HER2/Neu expression, ultimately resulting in tamoxifen resistance [10, 15, 16]. Our lab has shown that the loss of AIB1 in MCF-7 (ER-positive) breast cancer cells inhibits IGF-1 signaling, decreases IGF-1 receptor expression levels, and is rate limiting for IGF-1 induction of anchorage independent growth [11]. In sum, these observations demonstrate that AIB1 plays a significant role in several growth factor-induced pathways, resulting in the amplification of breast cancer cell survival and proliferation.

HER2/Neu is a member of the epidermal growth factor receptor (EGFR) family. The HER2/Neu proto-oncogene is amplified and overexpressed in 20-30% of human breast cancers. The amplification of the HER2/Neu gene results in an increase in cell division and an elevated rate of cell growth [12]. Furthermore, the overexpression of HER2/Neu is correlated with a

negative prognosis [13] and shortened disease-free survival [14]. In patients, overexpression of the growth factor receptor HER2/Neu has been an important predictor of tamoxifen responsiveness. More specifically, patients with ER-positive breast cancers that are also overexpressing HER2/Neu are less responsive to tamoxifen [15]. Recent clinical data has shown that the overexpression of HER2/Neu in ER-positive patients activates both ER and the ER coactivator AIB1, thereby contributing to the agonistic properties of tamoxifen [10, 15, 16]. Trastuzamab, a monoclonal antibody targeted at the HER2/Neu receptor has proven to decrease the progression of cancer and improve survival. However, similar to tamoxifen, clinical data has shown that patients administered Trastuzamab alone or with other chemotherapies also develop resistance to the treatment, resulting ultimately in the recurrence of breast cancer growth. Interestingly, the mechanism of resistance and the role of AIB1 in these types of patients to both of these therapies are still unknown.

In this investigation I will breed the MMTV-Neu mice with the AIB1 knockout (-/-) mice to elucidate the role of AIB1 in HER2/Neu-induced mammary tumorigenesis. The MMTV-Neu mice develop focal mammary tumors due to the overexpression of HER2/Neu in the mammary epithelium [17]. Furthermore, the AIB1-/- mice display reduced mammary gland development, dwarfism and abnormal reproductive function [8]. I want to determine whether the loss of AIB1 in MMTV-Neu mice alters the mammary gland morphology in MMTV-Neu transgenic mice. I propose that the loss of AIB1 will increase the latency and decrease the incidence of HER2/Neu-induced tumors.

BODY

Task 1 in my statement of work required the generation of female transgenic mice with the following genotypes: MMTV-Neu/AIB1 wildtype (w/t), MMTV-Neu/AIB1 heterozygote (+/-), MMTV-Neu/AIB1 knockout (-/-), as well as control litters MMTV-Neu, AIB1 w/t, AIB+/- and AIB1-/- mice. After successfully setting up the breeding pairs, it was imperative to determine the expression levels of the transgene(s) being studied. Using Qiagen's DNeasy kit, I extracted tail DNA from each mouse 3 weeks after birth (at the time of weaning). Figure 1A shows PCRs for the AIB1+/-, AIB1-/- and AIB1 w/t mice. Due to the high homology between the Neu transgene and its mouse homologue, HER2, PCR could not be utilized to determine Neu expression. For that reason, I developed the more specific TaqMan real time-PCR assay (Figure 1B). The TaqMan probe and primers were designed to target a 22 nucleotide sequence of Neu in a region that is not homologous to HER2. Overall, the goal of coordinating the MMTV-Neu/AIB1-/- breedings and all of the proper control breedings has been fulfilled. Furthermore, the establishment of genotyping methods for each transgene will allow me to continue breeding large numbers of mice to attain a greater power for my 4-and 6-month time points.

Task 1 (b;1&2) in my statement of work discussed harvesting mammary gland (MG) #4 to study changes in ductal morphology across the different genotypes. At this stage of the study, I have mice at 4-months of age with the following genotypes (n=5 for each litter): AIB-/-, AIB1+/-, AIB1 w/t, MMTV-Neu/AIB1-/-, MMTV-Neu/AIB+/-, MMTV-Neu/AIB1 w/t. In figure 2 I show wholemounts from the AIB1 w/t, AIB1+/- and AIB-/-. Xu et al has published that the AIB1-/- mice used in this study display dwarfism and the retardation of mammary gland growth [9]. At the 4-month time point, I similarly observed an overall decrease in mammary gland size, ductal density and secondary ductal branching. Furthermore, when examining the hemotoxylin and eosin (H&E) stained slides, I observed a significant decrease in the number of ducts per field in the AIB1-/- and AIB1+/- mice relative to the AIB1 w/t mice. Lastly, as

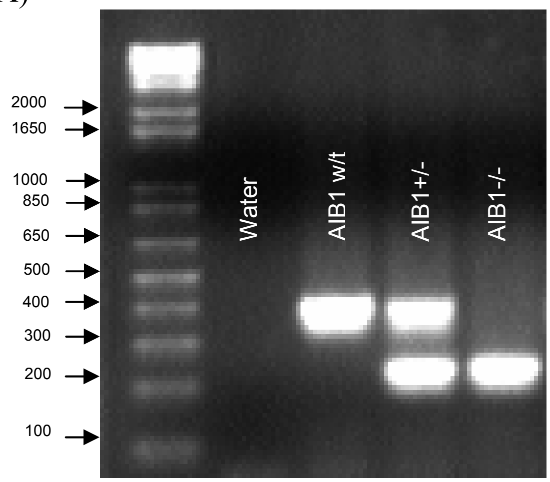
mentioned in the Task 1(b;3) the pancreas, liver, salivary glands and both MG#3s have been harvested and snap frozen for future protein expression studies.

I further examined the differences in the morphology and growth of the mammary glands from the MMTV-Neu/AIB1 w/t, MMTV-Neu/AIB1+/- and MMTV-Neu/AIB1-/- mice. It has been previously published that the overexpression of Neu in the MMTV Neu model revealed focal tumors at approximately 7-9 months [18]. The MMTV-Neu/AIB1 w/t mice displayed extensive ductal branching, and lateral budding along the primary and secondary ducts. Interestingly, as we hypothesized in the proposal, the MMTV-Neu/AIB1-/- showed a marked decrease in the overall ductal density, ductal branching and the absence of lateral budding along the primary and secondary ducts (Figure 3A). H&E stained AIB1-/- slides also showed a noticeable decrease in the overall number of ducts per field (Figure 3B). The MMTV-Neu/AIB1+/- mice also displayed the same phenotype; however the phenotype was not as dramatic (Figure 3A&3B).

Currently, I am in the process of investigating the 6-month time point in this study. My objective is to determine if the reduction and or knockdown of AIB expression in the AIB+/- and AIB1-/- mice increases the latency and decreases the incidence of tumor formation in the MMTV-Neu mice. Lastly, I am developing immunohistochemical assays for proteins that I believe may be responsible for the phenotype observed.

Figure 1: Genotyping was performed on DNA extracted from the tails of the mice. (A) AIB1 w/t, AIB1+/-, and AIB1-/- were identified with PCR analysis. (B) The presence of the Neu transgene was confirmed using quantitative real-time TaqMan PCR. DNA samples with a cycle number less than 21 were considered positive. Water was used in both genotyping procedures as a negative control.

A)



B)

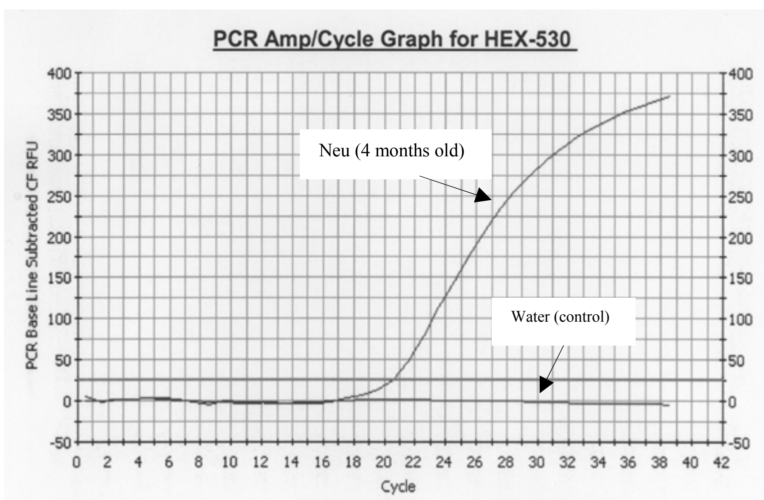
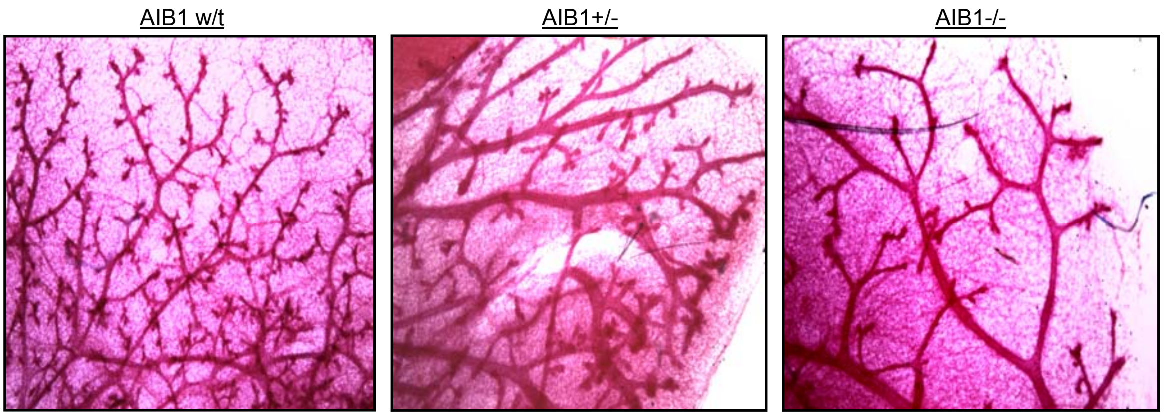


Figure 2: Representative (n=5) wholemount and H&E analysis of the #4 MGs harvested from AIB1 w/t, AIB1+/- and AIB1-/- mice. (A) Histological analysis of the wholemounts from the AIB1 w/t and the AIB1-/- reveals a dramatic decrease in ductal density and secondary branching in the AIB1-/- mice. (B) The H&E stained tissues displayed a reduction in the number of ducts per field in the AIB1-/- mice (4x magnification).

A)



B)

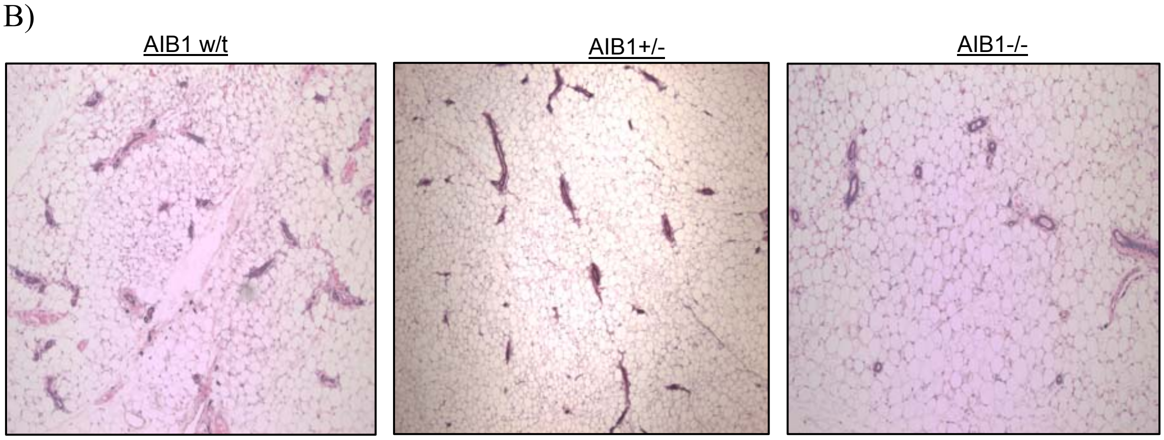
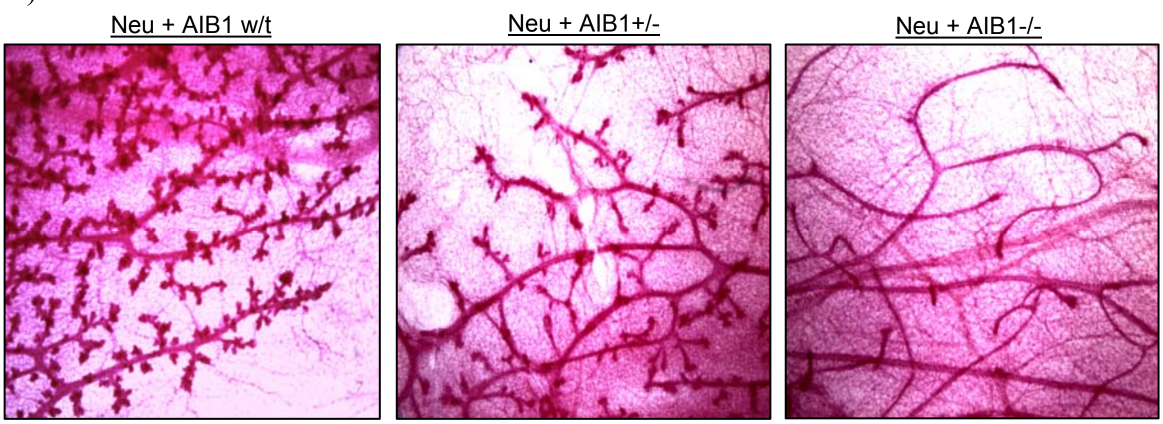
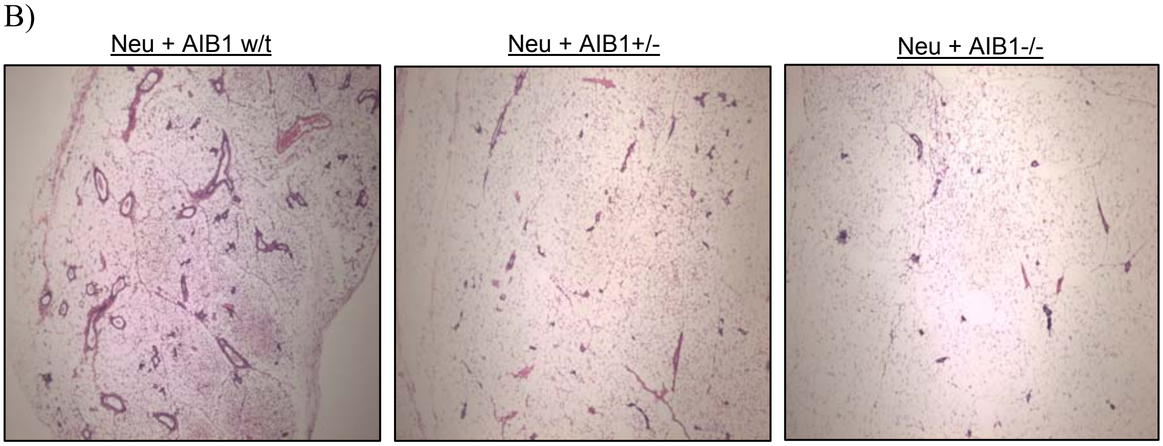


Figure 3: Representative (n=5) wholemount and H&E analysis of the #4 MGs harvested from MMTV-Neu/AIB1 w/t, MMTV-Neu/AIB1+/- and MMTV-Neu/AIB1-/- mice. (A) Histological analysis of the wholemounts from the MMTV-Neu/AIB1 w/t and the MMTV-Neu/AIB1-/- reveals a dramatic decrease in ductal density and secondary branching in the AIB1-/- mice. (B) The H&E stained tissues displayed a decrease in the number of ducts per field in the MMTV-Neu/AIB1-/- (4x magnification).

A)



B)



KEY RESEARCH ACCOMPLISHMENTS

- Generated the following female transgenic mice: MMTV-Neu (n=5), AIBw/t (n=5), AIB1+/- (n=5), AIB1-/- (n=5), MMTV-Neu/AIB1 w/t (n=5), MMTV-Neu/AIB1+/- (n=5), MMTV-Neu/AIB1-/- mice (n=5)

- Development of genotyping methods, specifically, real time PCR for the Neu transgene

- Histological analysis of wholemounts of the MMTV-Neu/AIB1 w/t (n=5), MMTV-Neu/AIB1+/- (n=5) and MMTV-Neu/AIB1-/- mice (n=5) at 4-months of age.

- Histological analysis of H&E stains of the MMTV-Neu/AIB1 w/t (n=5), MMTV-Neu/AIB1+/- (n=5) and MMTV-Neu/AIB1-/- mice (n=5) at 4-months of age.

REPORTABLE OUTCOMES

None thus far

CONCLUSIONS

The reduction of AIB1 in Neu overexpressing mice results in a decrease in lateral side bud formation, mammary gland ductal branching and ductal density. The preliminary data reported here implies that AIB1 is playing a significant role in Neu-mediated epithelial cell proliferation as evidenced by the reduction in the overall number of mammary gland ducts in the MMTV-Neu/AIB1-/- mice. I am currently collecting data from the 6-month time point, focusing specifically upon changes in cell proliferation (IHC: PCNA), changes in body weight across the different genotypes and the presence of preneoplastic lesions and palpable tumors.

ABBREVIATIONS

AIB1- Amplified in breast cancer 1
EGF- Epidermal growth factor
ER- Estrogen receptor
GR- Glucocorticoid receptor
GRIP1- Glucocorticoid receptor interacting protein 1
H&E- Hematoxillin and eosin
IGF- Insulin-like growth factor
IHC- Immunohistochemistry
MG- Mammary gland
MMTV- Mouse mammary tumor virus
PCNA- Proliferating cell nuclear antigen
PCR- Polymerase chain reaction
PPAR- Peroxisome proliferator-activated receptor
RAR- Retinoic acid receptor
rtTA- Reverse tetracycline transactivator
SRC- Steroid receptor coactivator

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